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Chapter 1

Strain-Specific Allele Loss: An Important Clue to Tumor Suppressors Involved in Tumor Susceptibility

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1. Introduction

Development of tumors is controlled by multiple genes such as cellular oncogenes and tumor suppressors activated or inactivated by somatic mutations and/or epigenetic mechanisms. Tumor development is also controlled by heritable factors as well as environmental factors, i. e., diet, oxidative stress and sustained inflammation, as reviewed by a large number of recent reports [1-12]. Both heritable and environmental factors are important targets for clinical controls and prevention of cancers.

Heritable factors underlying cancer risks have been identified in familial cancer-prone pedigrees. In the pedigree members, tumors develop in a Mendelian dominant inheritance fashion. Breast cancer 1, early onset (*BRCA1*) encoding a nuclear phosphoprotein that plays a role in maintaining genomic stability is one of the heritable cancer risk factors hitherto identified. Women bearing a mutated *BRCA1* allele are at high risk for both breast and ovarian cancers through their lifespan. According to the recent estimations, average cumulative risks in *BRCA1*-mutation carriers by age 70 years are 65% (95% confidence interval 44%–78%) for breast cancer and 39% (18%–54%) for ovarian cancer [13]. Thus, disease penetrance is incomplete, albeit rather high, in the mutated-*BRCA1* carriers. The *BRCA1* gene maps to human chromosome 17q21, where frequent loss of heterozygosity (LOH) is observed in both familial and sporadic breast cancers. Although tumors developed in the *BRCA1*-mutation carriers are homozygous for the defective *BRCA1* allele via LOH mechanisms, sporadic cases rarely show mutation in the *BRCA1* gene [14]. The *BRCA1* gene may rather undergo inactivation via epigenetic mechanisms such as DNA methylation in sporadic tumors.

Unlike the *BRCA1* case, tumor susceptibility is expressed in a non-Mendelian inheritance manner, because multiple genes with incomplete penetrance participate in the phenotype. Moreover, tumor susceptibility alleles may occasionally express genetic interaction, i. e.,

epistasis that hides or enhances the effect of some alleles at some susceptibility loci with the effect of other alleles at other susceptibility loci [15]. Despite growing number of association studies localizing tumor susceptibility loci exploiting SNPs in humans [16, 17], validation of these loci in human population with miscellaneous variations in the genetic background might be an intractable task.

Several strains of mice with different susceptibility to lymphomagenesis so far reported might be useful in the study of tumor susceptibility. Using genetic crosses between BALB/cHeA (refer to as BALB/c, hereafter) and STS/A (refer to as STS) mice with different tumor susceptibility, and between the BALB/c and recombinant congenic CcS/Dem strains of mice with 12.5% STS and 87.5% BALB/c allele in the genome, we mapped three loci controlling susceptibility to radiation-induced apoptosis of thymocytes to chromosomes 16, 9 and 3 [18, 19], and two loci for susceptibility to lymphomagenesis to chromosome 4 [20]. We identified the protein kinase, DNA activated, catalytic polypeptide (*Prkdc*) as a candidate for the apoptosis susceptibility gene mapped to chromosome 16, which was also associated with susceptibility to radiation lymphomagenesis [21]. As indicated by our studies, susceptibility to apoptosis as well as lymphomagenesis is controlled by multiple genes. To analyze the effect of one gene involved in such multigenic traits, congenic animals are ordinarily used. We are currently analyzing the genes controlling susceptibility to lymphomagenesis on chromosome 4 by the use of congenic animals.

In this chapter, we initially review recent advances in the research of tumor susceptibility, in particular, susceptibility to radiation lymphomagenesis in mice, and show that two loci controlling radiation lymphomagenesis map to chromosome 4. Then, we show that two types of allele loss, i. e., loss common to lymphoma and parental strain-specific loss, occur in radiation-induced lymphomas from various F_1 hybrids between strains with different lymphoma susceptibility. We show that LOH on chromosome 4 in F_1 hybrids between BALB/c and STS occurs in a strain-specific manner and exhibits a bias towards the STS allele loss. At the close, by exploiting congenic strains of mice containing different segments of chromosome 4 from the donor strain STS on the BALB/c background, we present a concordance between the allele loss region and a lymphoma susceptibility locus area on chromosome 4, where the BALB/c mouse harbors a hypomorphic allele of *Cdkn2a*. Significance of the strain-specific allele loss in probing tumor susceptibility loci will be discussed.

2. Mouse strain difference in susceptibility to radiation-induced lymphomagenesis

In laboratory strains of mice irradiated by ionizing radiation according to a well-established protocol, development of lymphomas starts around three months after the exposure to radiation and is terminated around ten months. Radiation-induced lymphomas are mostly of thymic origin. Several laboratory strains of mice such as BALB/c and C57BL reside in *Mus musculus musculus*, and are known to be highly susceptible to radiation-induced lymphomagenesis, while other strains STS and MSM/Ms (refer to as MSM) are not [22, 23]. The BALB/cHeA and STS/A strains of mice are originally provided by Dr. J. Hilgers at the Netherlands

Cancer Institute [22], and maintained more than twenty generations at the animal facility of Osaka Prefecture University. The BALB/cHeOpu mouse is the direct descendant of the BALB/cHeA mouse [20]. The MSM/Ms strain of mice belongs to a subspecies *Mus musculus molossinus*. Its progenitor was trapped in Mishima-city, Shizuoka, Japan and established as an inbred strain at the National Institute of Genetics (Mishima, Japan). Mice were exposed to 4 x 1.7 Gy of X rays using a well-established protocol for radiation-induced lymphomagenesis. Tumor development was observed during one year. The results were summarized in Table 1. BALB/cHeA mice developed lymphomas at high frequency (33/43, 77%), while STS/A mice develop tumors at less than 10% of frequencies [22]. The onset of tumor development was around three months in both strains. On the other hand, one of 30 MSM/Ms mice developed lymphoma with more than ten months of latency. Lymphomas occurred at high frequency (30/35, 86%) in BALB/cHeOpu mice subjected to X-ray irradiation using the same protocol. Thus, the pattern of tumor development in BALB/cHeOpu mice showed good concordance with that in their progenitor BALB/cHeA mice [20].

Strain of mice	Number of irradiated[a]	Number of affected (%)[b]	Reference
BALB/cHeA	43	33 (77%)	[22]
BALB/cHeOpu	35	30 (86%)	[20]
STS/A	60	5 (8%)	[22]
MSM/Ms	30	1 (3%)	[23]

[a]Only females.

[b]Cumulative incidence of lymphomas within one year in BALB/cHeA, STS/A and MSM/Ms, and within ten months in BALB/cHeOpu.

Table 1. Strain difference in susceptibility to radiation lymphomagenesis

3. Current status of the studies on tumor susceptibility in mice

Numerous tumor susceptibility loci have been mapped by analyzing genetic crosses between strains of mice exhibiting different tumor susceptibility [17]. Several genes responsible for tumor susceptibility have been identified, some of which are validated by supporting evidences: *Pla2g2a* encoding phospholipase A2, group IIA (platelets, synovial fluid), for the modifier of *Min1* (APC^{Min})-induced intestinal tumors (*Mom1*) identified in the distal portion of chromosome 4 [24–26]; cyclin-dependent kinase inhibitor 2A (*Cdkn2a*) encoding a tumor suppressor p16, for pristen-induced plsmacytoma resistance1 (*Pctr1*) mapped in the middle of chromosome 4 [27, 28]; protein tyrosine phosphatase, receptor type, J, (*Ptprj*), for susceptibility to colon cancer 1 (*Scc1*) on chromosome 2 [29, 30]. LOH occurs at *PTPRJ*, the human homolog of mouse *Ptprj* (*Scc1*), in the early stage of human colorectal cancer [31]. Hence, *PTPRJ* may play a role in tumor suppression in humans. The biological function of *Pla2g2a* (*Mom1*) differs from other tumor susceptibility genes so far identified. *Pla2g2a* plays a role in physiological processes such as anti-bacterial defense, inflammation and eicosanoid generation, which are preferable targets of medical controls for cancer prevention.

Despite the availability of strains of mice with obvious difference in susceptibility to radiation lymphomagenesis, it is much difficult to analyze such traits as to be expressed in a binominal fashion (tumor-free survivals of animals after exposure to radiation). However, there is one successful case: a suggestive linkage near D4Mit12 at 57.8 centimorgan (cM) position on chromosome 4 with susceptibility to radiation lymphomagenesis, which was detected in the genetic cross between BALB/c and MSM, is confirmed by exploiting congenic mice with the MSM allele at D4Mit12 on the BALB/c background [32, 33]. Because BALB/c mice had a hypomorphic allele at the *Mtf1* locus, they reported the metal-responsive transcription factor-1 (*Mtf1)* gene as the candidate gene for the susceptibility locus near D4Mit12 [32, 34]. *Mtf1* activates expression of metallothionein I and II genes as well as gamma-glutamylcysteine synthetase, a key enzyme for glutathione biosynthesis, and metallothionein and glutathione are involved in detoxification processes, such as scavenging reactive oxygen intermediates generated by ionizing radiation. Reduced reactivity of Mtf-1 retains an increased level of ROS in the BALB/c thymus [35].

4. Mapping of lymphoma susceptibility loci on mouse chromosome 4 using genetic crosses between BALB/c and STS strains of mice

We so far showed that the protein kinase, DNA activated, catalytic polypeptide (*Prkdc*) gene was a candidate for the apoptosis susceptibility gene on chromosome 16, and also responsible for susceptibility to radiation lymphomagenesis [21]. DNA-PK is a key enzyme for DNA double-stranded-break repair as well as V(D)J recombination of T- and B-cell receptors. Because BALB/c mice carry a *Prkdc* variant allele that causes lower DNA-PK activity, resultant hypersensitivity to radiation may raise frequency of cell death in the thymus and promote lymphomagenesis possibly via illegitimate recombination mechanisms. However, strain difference between BALB/c and STS in susceptibility to radiation lymphomagenesis has not been fully explained by the variations in *Prkdc*. According to M. Okumoto *et al.* [22, 23], cumulative incidence of lymphomas in (BALB/c x STS)F_1 exposed to 4 x 1.7 Gy of X-ray irradiation was in between those in parental BALB/c and STS, while (BALB/c x MSM)F_1 developed lymphomas at high frequency similar to BALB/c. The data suggest that strain difference in tumor susceptibility is controlled by multiple genes that influence onset, latency and frequency of tumorigenesis.

Previously, M. Okumoto *et al.* reported a suggestive linkage of susceptibility to radiation-induced lymphomagenesis, named lymphoma resistance *(Lyr*) (Mouse Genome Informatics, MGI: 96893) in the middle area of chromosome 4 using a series of recombinant inbred (RI) CXS strains of mice whose genome was constituted of 50% STS and 50% BALB/c genes [36]. It is worthwhile to test whether the *Lyr* locus is segregated in a genetic cross using BALB/c and STS. We performed genome-wide screen for microsatellite markers linked to lymphoma susceptibility using siblings from (BALB/c x STS)F_1 backcrossed to BALB/c or STS. We detected significant linkage disequilibrium in the middle area of chromosome 4 by the use of 219 siblings from (BALB/c x STS)F_1 backcrossed to BLB/c [20]. No significant linkage was detected by using another backcross. The primary locus with a conspicuous effect existed in an approximately 10 cM segment spanning D4Mit302 (37.6 cM) and D4Mit255 (48.5 cM) in the middle range of

chromosome 4 (χ^2=19.3, genome-widely corrected p=0.0075). This locus was likely identical to the *Lyr* locus localized between tyrosinase-related protein 1 (*Tyrp1*) (38 cM) and interferon alpha (*Ifna*) (42.6 cM) [36]. The secondary locus with a weaker effect was detected near D4Mit17 (χ^2=16.0, genome-widely corrected p=0.034), a marker approximately 10 cM proximal to D4Mit302. The STS allele at these loci was associated with resistance to lymphomagenesis. *Mtf1*, a candidate susceptibility gene for radiation lymphomagenesis so far identified by other investigators, is located near D4Mit12 (57.8 cM), more than 10 cM distal to the critical regions containing these loci [32, 33]. Effect of *Prkdc*, which we identified as a lymphoma susceptibility gene by exploiting congenic mice [21], was not detected in tumor-free survivals in these crosses.

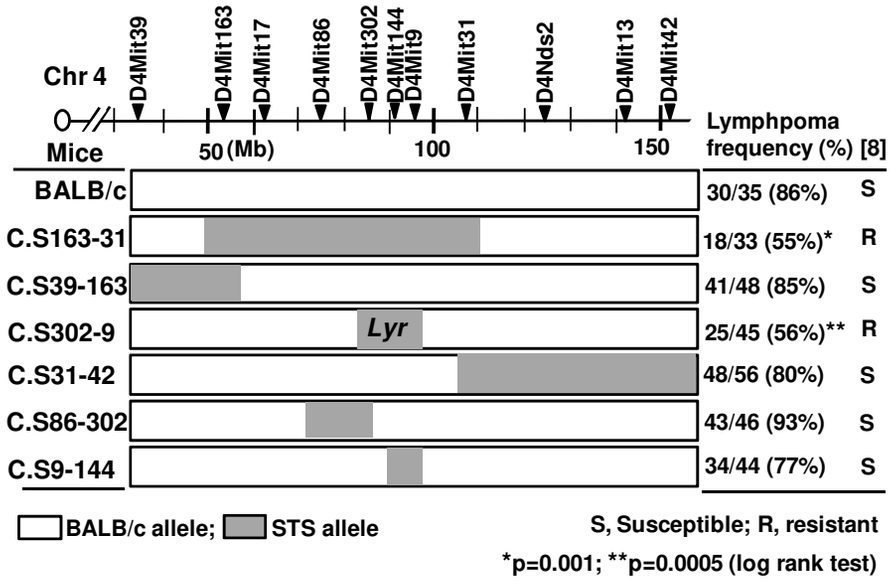

Figure 1. The *Lyr* locus exists between D4Mit302 and D4Mit144 on chromosome 4.

To narrow down the tumor susceptibility gene regions, we generated congenic strains of mice with different portions of STS-derived chromosome 4 on the BALB/c background by back-crossing (BALB/c x STS)F_1 mice to the BALB/c. Establishment of the congenic lines was facilitated by positive and negative selections with typing of microsatellite markers on chromosome 4 and markers distributed in the whole genome [20]. Because the *Lyr* locus was so vicinal (10 cM distance, approximately) to the secondary locus, we selected several strains with or without the STS allele at the critical markers D4Mit17, D4Mit302 and other markers near these markers, and compared their tumor-free survivals with that of BALB/cHeOpu exposed to X-ray irradiation (data shown in Table 1). A part of the results in [20] is represented in Figure 1. In this figure, the strain names of the C.S congenic mice are abbreviated by hyphened two Arabic numbers that represent STS allele-bearing microsatellite (Mit) markers at the proximal and distal end of the chromosomal segment. For instance, C.S163–31 represents a congenic line with the STS allele in the segment spanning D4Mit163 and D4Mit31. The order and megabase (Mb) positions of the markers are indicated by arrowheads on chromosome 4, which is represented by a line at the top of the figure. The primary lymphoma susceptibility

locus *Lyr* exists between D4Mit302 (85.2 Mb) and D4Mit9 (94.7 Mb). Although the secondary locus was not detectable by a simple comparison of the tumor-free survival of congenic lines with that of BALB/c, linkage was reconfirmed by crossing congenic lines (data not shown here).

5. Loss of heterozygosity (LOH) in radiation-induced lymphomas from various F_1 hybrids: common loss and cross-dependant loss

Tumor suppressors frequently undergo loss of heterozygosity (LOH) in a variety of tumors in humans and mice. We previously reported that frequent LOH (more than 20%) occurred on chromosomes 4, 12 and 19 in radiation-induced lymphomas from (BALB/c x STS) F_1 mice, with incidences 27% (20 of 74 lymphomas), 57% (42 of 74 lymphomas) and 50% (37 of 74 lymphomas) on chromosomes 4 (at D4Mit31), 12 (at D12Mit17) and 19 (at D19Mit11), respectively [37] (Table 2). Importantly, STS allele-specific loss occurred on chromosome 4. The bias was confirmed using reciprocal F_1 hybrids between BALB/c and STS [37].

Mice[a]	Number of tumors	Chr	Marker (Mb)[b]	LOH (%)	Reference
(CXS)F_1	74	4	D4Mit31 (106.8)	20 (27%)	[37]
		12	D12Mit17[c]	42 (57%)	
		19	D19Mit11 (42.5)	37 (50%)	
(SXM)F_1	20	4	D4Mit54 (137.4)	5 (25%)	[39]
		12	D12Mit233 (109.5)	12 (60%)	
(CXM)F_1 [d]	81	12	D12Mit181 (110.0)	53 (65%)	[40]
		16	D16Mit122 (74.5)	38 (45%)	

[a]Abbreviations used are BALB/c, C; MSM, M; STS, S.

[b]Megabase (Mb) positions of markers are according to Mouse Genome Informatics (MGI) 5.10.03. (http://www.informatics.jax.org/).

[c]Physical position not assigned.

[d]F_1 hybrids between BALB/c and MSM hemizygous for *Trp53*.

Table 2. LOH in radiation-induced lymphomas from various F_1 hybrids.

In these crosses, allele loss involved almost entire chromosomes 4 and 19, without showing any peaks in LOH frequencies. Cytogenetic analysis showed that allele loss in such large areas was not caused by chromosomal deletion, but ascribable to mitotic recombination [38]. In lymphomas from (STS x MSM)F_1 mice, LOH occurred on chromosomes 4 and 12 with incidences 25% (5 of 20 lymphomas) and 60% (12 of 20 lymphomas) on chromosome 4 (at D4Mit54) and chromosome 12 (at D12Mit233), respectively [39]. In these lymphomas, LOH on chromosome 19 was infrequent (1/20, 5% at D19Mit63). In radiation-induced lymphomas from (BALB/c x MSM)F_1 mice, allele loss frequently occurred on chromosomes 12 (53/81, 65% at D12Mit181) and 16 (38/81, 45% at D16Mit122) [40].

Interestingly, LOH on chromosome 12 commonly occurred in radiation-induced lymphomas from these three F_1 hybrids, while LOH frequencies on chromosomes 4 and 19 markedly varied. Frequent LOH was detected on chromosome 4 in lymphomas from (STS x MSM)F_1 mice, but not (0/20 at D4Mit13) in lymphomas from (BALB/c x MSM)F_1 mice [40]. LOH on chromosome 19 was infrequent (0/20 and 1/20, at D19Mit63 and D19Mit123) in lymphomas from (STS x MSM)F_1 mice. In the context of LOH on chromosome 19, results were similar in lymphomas from the (BALB/c x MSM)F_1 hybrid. Thus, LOH on chromosomes 4 and 19 occurred in a cross-dependent manner. This suggests that LOH frequencies on these chromosomes are controlled by genetic interaction, possibly between putative tumor suppressors, the locations of which are indicated by LOH, and by genetic variations in the background. Moreover, the situation of LOH on chromosome 4 is somewhat different from that on chromosome 19. We present allele loss frequencies at several markers on chromosome 4 in these lymphomas in Table 3.

Mice[a]	Number of tumors	Marker (Mb)[b]	LOH (%)	References
(CXS)F_1	47	D4Mit17 (63.0)	14 (30%)	[37]
		D4Mit9 (94.7)	14 (30%)	
		D4Mit13 (142.0)	14 (30%)	
(SXM)F_1	20	D4Mit9 (94.7)	1 (5%)	[39]
		D4Mit54 (137.4)	5 (25%)	
(CXM)F_1 [c]	20	D4Nds2 (124.4)	0 (0%)	[40]
		D4Mit13 (142.0)	0 (0%)	
(CXM)F_1	43	D4Mit9 (94.7)	3 (7%)	Unpublished data
	51	D4Mit13 (142.0)	4 (8%)	[41]

[a]Abbreviations used are BALB/c, C; MSM, M; STS, S.

[b]Megabase (Mb) positions of markers are according to Mouse Genome Informatics (MGI) 5.10.03. (http://www.informatics.jax.org/).

[c]F_1 hybrids between BALB/c and MSM hemizygous for *Trp53*.

Table 3. Variation of LOH frequencies at microsatellite markers on chromosome 4 in radiation-induced lymphomas from various F_1 hybrids.

Notably, LOH frequency at D4Mit9 was reduced compared to that at D4Mit54, a marker in the proximity of D4Mit13 and approximately 43 Mb distal to D4Mit9, in lymphomas from (STS x MSM)F_1 hybrid mice. Using lymphomas from (BALB/c x MSM)F_1 mice, we reconfirmed that allele loss at markers D4Mit9 and D4Mit13 on chromosome 4 was very infrequent (3/4 and 4/51 [41], respectively). Because D4Mit9 is located very close to cyclin-dependent kinase inhibitor 2A (*Cdkn2a*) encoding tumor suppressors p16 and p19Arf, *Cdkn2a* is excluded as the putative tumor suppressor for lymphomagenesis in the (STS x MSM)F_1 and (BALB/c x MSM)F_1 backgrounds. Frequent LOH on chromosome 4 and 19 were also reported by other investigators in lymphomas from (C57BL/6JxBALB/cJ)F_1 and (C57BL/6J x RF/J) F_1 hybrid mice [42, 43]. According to the data in [43], strain-specific allele elimination is not found in the LOH on chromosome 4 from (C57BL/6JxBALB/cJ)F_1 mice.

The LOH frequencies at markers on chromosome 12 formed a sharp peak near telomere [41], and a putative tumor suppressor B cell leukemia/lymphoma 11B (*Bcl11b*) was later cloned from the peak [44]. The *BCL11B* tumor suppressor is also involved in human T cell acute lymphoblastic lymphomas [45]. Some of the lymphomas used for the genome-wide screen of LOH were generated in *Trp53* hemizygous (BALB/c x MSM)F_1 mice [40]. Because LOH frequencies at markers on chromosome 16 were markedly varied depending on the status of *Trp53* in (STS x MSM)F_1 mice [39], the high frequency of the LOH on chromosome 16 observed in lymphomas from (BALB/c x MSM) F_1 mice may likewise be explained.

6. The STS allele-specific loss occurred in the Lyr region on chromosome 4

Allele loss on chromosome 4 was significantly biased towards loss of the STS allele in lymphomas from (BALB/c x STS)F_1 mice [37]. It is of interest to examine whether putative tumor susceptibility genes on chromosome 4, which we identified in different regions of chromosome 4, are associated with the strain-specific allele loss on chromosome 4 by using congenic strains of mice with various regions of chromosome 4 from the donor strain STS on the background strain BALB/c, namely the C.S congenic series. LOH was studied in lymphomas generated in (BALB/c x C.S163–31)F_1 and (BALB/c x C.S302–9)F_1 mice. Both C.S163–31 and C.S302–9 strains of mice showed resistance to lymphomagenesis as shown in Figure 1. The C.S163–31 strain harbors the STS allele at two tumor susceptibility loci, one locus near D4Mit17 and the other, *Lyr* in the D4Mit302–D4Mit9 segment. The results are shown in Table 4.

Frequent allele loss at markers in the chromosome 4 segments was detected in lymphomas from (BALB/c x C.S163–31)F_1 (cross A) and (BALB/c x C.S302–9)F_1 (cross B) with incidences 11/34 (32%) and 10/34 (29%), respectively. The LOH frequencies in these F_1 hybrids were concordant with the original data in (BALB/c x STS)F_1 ([37] in Table 3). The STS-allele loss ratios were 9/11 (D4Mit302) and 10/11 (D4Mit9) in the cross A; 8/10 (D4Mit302) and 9/10 (D4Mit9) in the cross B. Because the STS-allele loss occurred with similar ratio in both crosses, we combined the data from crosses A and B (presented as A + B in Table 4). Analysis of the combined ratios 17/21 (D4Mit302) and 19/21 (D4Mit9) indicate that the distortions are significant at both markers D4Mit304 and D4Mit9 ($\chi 2$ values were 8.0 and 13.7, $p<0.005$, degree of freedom = 1, respectively). The data indicating the STS-allele specific loss (D4Mit31) in lymphomas from reciprocal (BALB/c x STS)F_1 and (STS x BALB/c)F_1 hybrids are also presented ([37] in Table 4). Thus, the skewed allele loss that was originally observed in a wide area of chromosome 4 in (BALB/c x STS)F_1 and (STS x BALB/c)F_1 hybrids is reproducible in the limited segments of the STS-derived chromosome 4. Our results suggest that tumor suppressor(s) associated with susceptibility to lymphomagenesis exist in the limited areas of chromosome 4. Since C.S39–86 mice carry the STS allele in the vicinity of D4Mit17, i. e., the secondary locus controlling susceptibility to lymphomagenesis, we further examined allele loss at markers D4Mit7 (67.7 Mb), a marker in the vicinity of D4Mit17, and D4Mit86 using 25 lymphomas from (BALB/c x C.S39–86)F_1 x BALB/c mice [20]. Allele loss at these markers was detected in only one of 25 tumors (less than 5%). In this case the BALB/c allele was lost. Hence, approximately 40 Mb of the D4Mit39–86 segment, to which the secondary locus for tumor susceptibility was

localized, was excluded from the skewed loss region. Analysis on congenic strains strongly suggest that the STS-strain specific loss is ascribable to the D4Mit302–D4Mit9 segment of chromosome 4, which harbors a putative tumor susceptibility gene *Lyr*.

Mice[a]	Number of tumors	Marker (Mb)[b]	LOH (%)	S loss	C loss
A. (C x C.S163–31)F_1	34	D4Mit17 (63.0)	11 (32%)	9	2[c]
		D4Mit302 (85.2)	11 (32%)	9	2[c]
		D4Mit9 (94.7)	11 (32%)	10	1[c]
		D4Mit31 (106.8)	11 (32%)	10	1[c]
B. (C x C.S302–9)F_1	34	D4Mit302 (85.2)	10 (29%)	8	2[d]
		D4Mit9 (94.7)	10 (29%)	9	1[d]
A + B	68	D4Mit302 (85.2)	21 (31%)	17	4
		D4Mit9 (94.7)	21 (31%)	19	2
(C x S)F_1	39	D4Mit31 (106.8)	11 (28%)	10	1[e]
(S x C)F_1	35	D4Mit31 (106.8)	9 (26%)	7	2[e]
(C x S)F_1 + (S x C)F_1	74	D4Mit31 (106.8)	20 (27%)	17	3[e]

[a]BALB/c and STS mice are abbreviated as C and S.

[b]Megabase (Mb) positions of markers are according to Mouse Genome Informatics (MGI) 5.10.03. (http://www.informatics.jax.org/)

[c]Unpublished data.

[d] Data in [20].

[e]Data in [37].

Table 4. LOH at markers on chromosome 4 in lymphomas induced by radiation in F_1 hybrids between BALB/c and STS or C.S congenic lines.

In the *Lyr* region between D4Mit302 and D4Mit144, three known tumor suppressors *Cdkn2a*, cyclin-dependent kinase inhibitor 2B (*Cdkn2b*) encoding p15INK4B and methylthioadenosine phosphorylase (*Mtap*) exist (Figure 2). Involvement of *Cdkn2a* and *Cdkn2b*, specifically in acute lymphoblastic lymphomas (ALL) in humans and thymic lymphomas in mice has been reported [46–49]. Mtap is a key enzyme in purine and polyamine metabolism and regulation of transmethylation reactions and frequently inactivated in human tumors such as lymphomas by large homozygous deletion of the 9p21 region [50]. Since these deletions inactivate *CDKN2A/ARF* and *CDKN2B* as well as *MTAP* [51], it has been hypothesized that loss of *MTAP* in tumors is a result of co-deletion. However, a recent study showed that mice heterozygous for the targeted *Mtap* gene were affected with T-lymphocyte hyperproliferation followed by T-cell lymphomas late in their lives [52]. In these lymphomas, as shown by the study, expression of *Mtap* was markedly reduced, while expression of *Cdkn2a* was not. The results suffice the criteria for tumor suppressors, indicating that *Mtap* is a candidate tumor suppressor distinct from *Cdkn2a*. It has also been reported that the *Cdkn2b* gene is particularly inactivated by allele loss and hypermethylation of the remainder allele in radiation-induced lymphomas in mice [53]. BALB/c mice carry a hypomorphic variant allele at *Cdkn2a*, which is shown to be

causative in the sensitivity to plasmacytomagenesis [28]. STS mice and most of strains other than BALB/c have the wild-type allele at the *Cdkn2a* locus ([28], DNA sequences we confirmed). Although *Cdkn2a* is a potential candidate for tumor susceptibility gene *Lyr*, *Cdkn2b* and *Mtap* are at present not ruled out as candidates for the tumor susceptibility gene. Analysis for allele loss, sequences and expression levels of these tumor susceptibility genes in BALB/c and C.S302–9 congenic mice is currently underway.

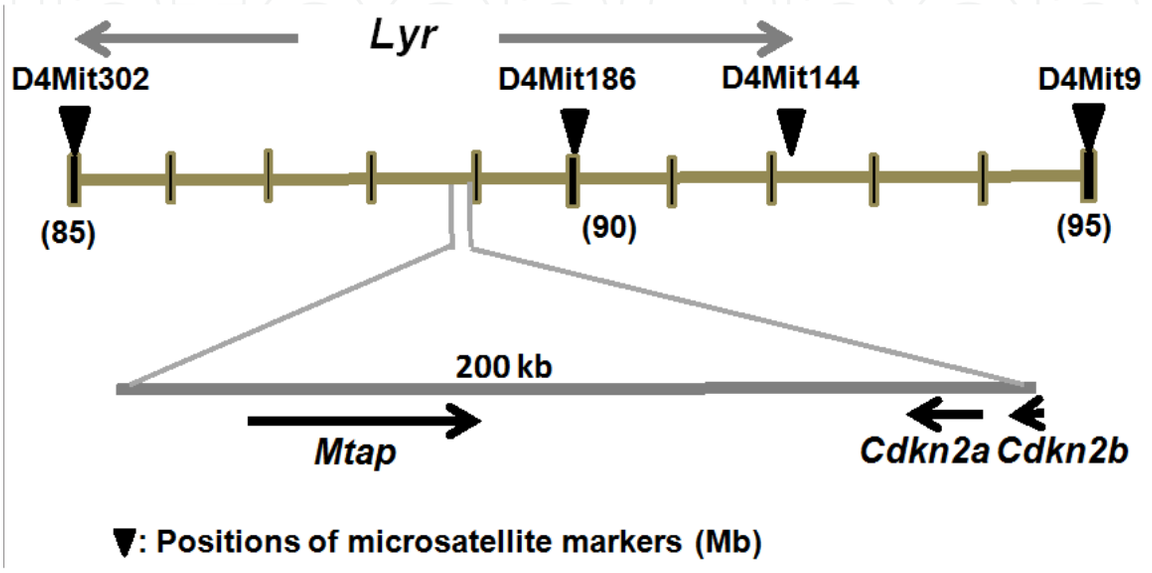

Figure 2. Locations of tumor suppressors in the lymphoma susceptibility *Lyr* gene region.

7. Conclusion

Frequent LOH occurs on chromosomes 4, 12 and 19 in radiation-induced lymphomas from various F_1 hybrid mice. These allele losses are classified into two groups: common loss and cross-dependent loss. The putative tumor suppressor harbored in common loss on chromosome 12 might be a key player in radiation-induced lymphomgenesis. Cross-dependent allele loss such as those on chromosomes 4 and 19 reflects genetic interaction between tumor suppressors harbored in the LOH region and the genetic background. BALB/c and STS strains of mice are susceptible and resistant to radiation-induced lymphomagenesis, respectively. Allele loss occurs on chromosome 4 in approximately 30% of lymphomas induced by radiation in (BALB/c x STS)F_1 mice and shows preferential loss of the STS allele. Our analysis of congenic lines with various portions of STS-derived chromosome 4 on the BALB/c background shows a link between the skewed LOH and the tumor susceptibility *Lyr* locus, where tumor suppressors *p16Ink4a/Arf*, *p15Ink4b* and *Mtap* genes are localized. Although the *Lyr* gene is as yet unidentified, *p16Ink4a/Arf* may be one of the potential candidates. Studying cross-specific LOH and distorted allele loss may lead to better understanding of variable pathways of radiation lymphomagenesis.

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